

The Gametophyte and Young Sporophyte of *Athyrium esculentum*

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Athyrium esculentum (Retz.) Copel. is one of the species of ferns that has suffered the most in nomenclature, having been by different authors attributed to at least eight different genera (*Hemionitis*, *Diplazium*, *Asplenium*, *Anisogonium*, *Microstegia*, *Callipteris*, *Digrammaria*, and *Gymnogramme*). It extends from Polynesia to India, growing as a straggling weed in marshy, or just moist, areas which are not necessarily shaded. *A. esculentum* lacks the elegance of most other species of *Athyrium* and consequently is not favoured as an ornamental fern, although it is comparatively easy to cultivate and is one of the most important of ferns as human food. The tender leaves of the plant are used as a vegetable in preparing tasty salads, pickles, etc.

Comparatively little is known regarding the gametophyte of *Athyrium esculentum* or for that matter any species of *Athyrium*. In view of this, spores were collected from plants growing at the National Botanic Gardens (Lucknow) and sown in September, 1955, on sand beds irrigated from below and maintained in a glass house. The technique followed is as described earlier (Kachroo & Nayar, 1953; Nayar, 1954).

The spores of *A. esculentum* (Figs. 2, 3) are bilateral,¹ anisopolar, with a single linear short proximal laesura, of medium size, monolete, concavo-convex in equatorial view, with one of the equatorial ends narrower than the other and with a brown scabrate exine (having small irregular elevated patches). The exine pattern (Fig. 1) is discernible only in acetolysed and bleached preparations. The average size of the spores is P 29.16 μ , E₁ 44.00 μ and E₂ 30.24 μ . The size variations are: P 25.00 to 32.50 μ , E₁ 39.50 to 50.50 μ and E₂ 25.00 to 36.00 μ .

In culture the spores germinate within a week. The first

¹ The acetolysis method (Erdtman, 1952) was used in the study of spores and the terminology used in spore description is after Harris (1955).

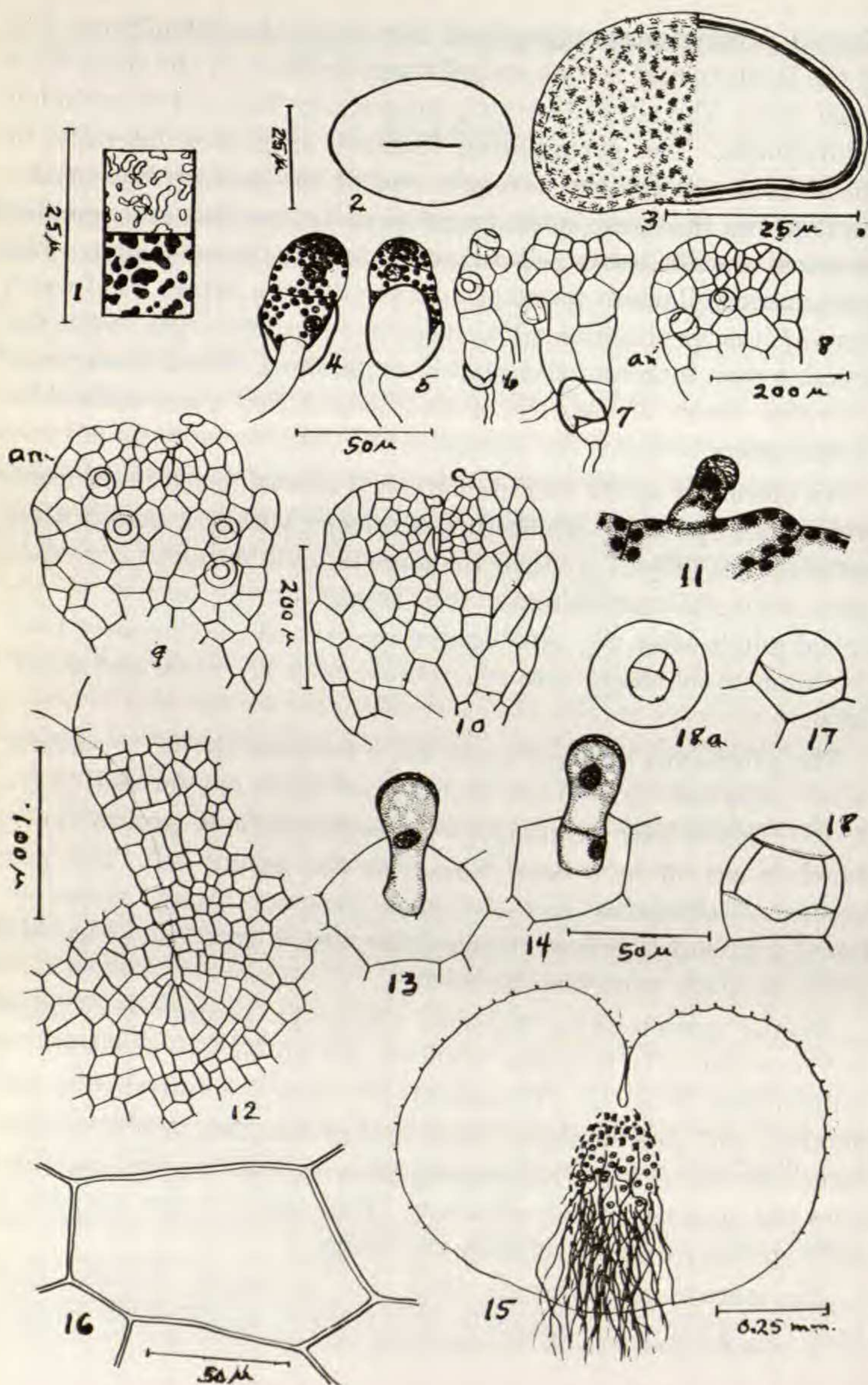
rhizoid protrudes as a papillose structure, the exine rupturing at the laesura, and is soon cut off from the body of the spore by a basal wall. The rhizoid in early stages may have a few included chloroplasts. The germinating filament originates laterally to the rhizoid towards the narrower end of the spores (*Figs. 4, 5*). As it grows the exine splits longitudinally into two and remains attached to the basal cell till very late in development. The germinating filament becomes 3 to 4 cells long before the formation of the prothallial plate begins (*Fig. 6*). The cells are broader than long and densely chlorophyllous. Rhizoids originate laterally. Some of the cells of the filament may form antheridia at this stage.

An obconical apical meristematic cell is established in the usual way and a spatulate prothallus is formed within a month after germination (*Fig. 7*). Soon the gametophyte develops a cordate apex with the meristematic cell lodged at the bottom of the apical notch (*Fig. 8*). Antheridia are formed continuously from the filamentous stage onwards and are both marginal and superficial.

The prothallus remains naked till it becomes distinctly cordate, when marginal unicellular, club-shaped hairs are formed (*Figs. 9, 10*). Each hair originates as a mammilliform protuberance, which is cut off by a basal wall from the parent cell. The protuberance elongates and the apex becomes highly vacuolate. Soon, a greenish-yellow extracellular cap is secreted (*Fig. 11*), which in older hairs may be shed.

As the gametophyte becomes distinctly cordate the apical meristematic cell becomes replaced by an apical meristem of conical cells (*Fig. 12*). Formation of a midrib is initiated by two months' old gametophytes and archegonia are produced continuously thereafter. Superficial hairs resembling the marginal ones but sometimes two cells long (*Figs. 13, 14*) are also developed sparsely over the midrib and wings.

The mature gametophyte (*Fig. 15*) is cordate, broader than long (ca. 10 mm in diameter) with a deep apical notch usually



overlapped by the lateral lobes and with a prominent midrib bearing sex organs on the ventral surface. The wing cells are uniformly thin-walled (*Fig. 16*) and densely chlorophyllous. The sex organs are of the usual type in higher ferns. Antheridia are globular and generally sessile (*Figs. 17, 18*). Occasionally the opercular cell is divided into two, three (*Fig. 18a*), or four cells. In liberating the sperms the opercular cell is entirely thrown off.

Gametophytes three months old produce sporelings. Generally only one sporophyte is formed per gametophyte (*Fig. 19*). The juvenile leaves are of the midribless type (terminology after Wagner, 1952b). The simplest cotyledonary leaf is cuneate (*Figs. 20, 21*) with a short petiole and a single vein forking equally twice. Their bases generally form an angle of less than 90° and the veinlets near the middle of the lamina run parallel to each other. The apex is usually truncate or shallowly notched.

Generally the second leaf (in some cases the first leaf itself) marks the next stage in development. It has a broader lamina with a distinct notch at the apex and a wider angle at the base. The veins fork three times (*Fig. 22*) with the branches towards the middle of the lamina more pronounced and sometimes forking once again in such a way as to give an appearance of pinnate branching (*Fig. 19-ii*). In such cases usually one side of the leaf is larger than the other (the right hand half in *Figs. 19-ii* and *22*).

The third stage is usually met with in the fourth or fifth leaf

Figs. 1-18. Spore- and gametophyte-morphology of *A. esculentum*. *Fig. 1.* L. O. pattern of the spore exine; *Fig. 2.* Equatorial view of spore; *Fig. 3.* Proximal polar view of spore showing laesura; *Figs. 4, 5.* Origin of the germinating filament; *Figs. 6-10.* Early stages in the development of the prothallus; *Fig. 11.* Marginal hair on mature prothallus; *Fig. 12.* Apex of mature prothallus showing meristem and adjoining tissue; *Figs. 13, 14.* Superficial hairs on mature prothallus; *Fig. 15.* Mature prothallus (diagrammatic); *Fig. 16.* One of the wing cells showing thickenings at corners; *Figs. 17, 18.* Stages in development of antheridium (dotted line represents surface pattern); *Fig. 18a.* Surface view of mature antheridium showing a divided cap cell.



or in weak individuals even later. The lamina broadens and the apex becomes elongated giving an oval shape to the leaf. The main vein entering the leaf base instead of dichotomising proceeds towards the tip as a midrib and gives off lateral veins alternately. The lower lateral vein on each side dichotomises once or twice, the branches towards the middle being longer than the others (*Figs. 19-iii, 23*). The transition to the midribbed stage is rather sudden and no intermediate stages have been observed. The leaf margin is wavy, the depressions corresponding with the spaces between vein tips.

In the fifth or sixth leaf the lamina broadens considerably and becomes trilobed (*Fig. 24*), the midrib and its upper branches occupying the middle lobe and the basal pair of lateral veins occupying the lateral lobes. The lateral veins develop in the same manner as the midrib. Just below the sinus on either side are formed the first areoles, by the basal adaxial tertiary veinlet of the lateral lobes joining with the basal secondary veins of the middle lobe or a branch of it. After joining, the fused vein proceeds towards the base of the sinus.

In later formed leaves the middle lobe becomes more pronounced and the basal secondaries of it begin forming the next pair of lobes with an areole at the base of each sinus (*Fig. 25*). The sinuses separating the first pair of lobes become deeper and almost reach the midrib making the leaf pinnatisect. Consequently the areole at the base of the sinus is not formed.

Further expansion of the lamina is by a pronounced increase in length of the leaf and formation of successive lateral lobes on

Figs. 19-39. Morphology of the juvenile leaves of *A. esculentum*. *Fig. 19.* Gametophyte with attached sporeling (growing apex of the sporeling not shown; *i, ii, iii*—the first, second and third leaves); *Figs. 20-26.* Leaf succession in the young sporophyte; *Fig. 27.* Portion of adult lamina showing venation pattern (*mr*—midrib, *lv*—lateral vein); *Fig. 28.* Portion of margin of first leaf showing hairs (*h*—club-shaped hair, *ah*—acicular hair); *Fig. 29.* Superficial hair on first leaf; *Fig. 30.* Multicellular hair on the fourth leaf; *Figs. 31, 32.* Superficial hairs on the sixth leaf; *Figs. 33, 34, 35.* Hairs on the petiole of the seventh leaf; *Fig. 36.* Palea on the petiole of the same; *Figs. 37, 38, 39.* Hairs on the lamina of adult leaf.

either side of the midrib (*Fig. 26*). Though areoles are formed on both sides of the midrib connecting successive secondary veins, they do not form later as the lobes become separated. Instead, areoles of the same pattern are formed on either side of the secondary veins in the lobes. The margins of the lobes remain wavy. The leaves pass on from the pinnatifid to the pinnate condition by gradual deepening and broadening of the base of the sinuses, until the lamina is reduced to inconspicuous wings on the midrib (now the rachis) and finally to a deep green line lodged in an inconspicuous groove on the sides. The leaf-lobes (now the pinnae) develop a narrow stalk-like base and the venation becomes more complicated.

Increase in the size of the leaf is more marked in the longitudinal plane until an oblong deeply pinnatifid lamina is obtained. The midrib becomes grooved on the upper surface. The lateral lobes elongate, become oblong with almost parallel sides and a tapering apex. The secondary lateral veins of the lobes produce alternating tertiary branches which run obliquely to the secondary veins. The first formed tertiaries from nearby secondaries fuse to form a single vein which runs parallel to the secondaries for a short distance and ends blindly. As the leaf-lobe expands, more tertiaries are produced by each secondary vein and the lower ones fuse in pairs. The fusion vein from each basal pair of tertiaries while proceeding towards the margin fuses with successive tertiaries on either side, thus forming two regular rows of obliquely placed areoles between the nearby secondaries (*Fig. 27*). Tertiaries formed towards the tips of secondaries are free. The fusion veins above the last pair of areoles either end blindly below the marginal sinus of the lamina or in some cases fork just below the sinus, the branches running parallel to the sides of the sinus for some distance.

Once-pinnate leaves characterize the young plants of *A. esculentum* for quite a long time, and adult plants may revert to this leaf form under adverse conditions of growth. The bipinnate leaves of the adult plants are formed by the pinnae of the juvenile leaves undergoing the same pattern of development as

the main leaf itself.

The cotyledonary leaf bears unicellular, thin-walled, acicular hairs (*Fig. 28, ah*) all over the lamina and petiole. Mixed with them, unicellular club-shaped hairs (*Fig. 28, h; Fig. 29*) resembling those on the gametophyte, but without the caps, occur sparsely. The fourth leaf bears also club-shaped, uniseriate, multicellular hairs (*Fig. 30*) toward the base of the blade and on the petiole. These hairs are much bigger than the unicellular hairs and become pale brown when fully developed. The multicellular hairs become more numerous in succeeding leaves. The cell at the apex of the hairs becomes more prominent being globular, much bigger than other cells, and sharply marked off from the main body (*Figs. 31, 32, 33*).

The seventh or the eighth leaf is the first one to bear paleae. The multicellular, club-shaped hairs on these bear lateral unicellular glandular branches resembling gametophytic hairs (*Figs. 34, 35*). Later, the cells near the basal region of the hair expand and divide longitudinally, initiating the formation of a flattened base. The apical region in all cases remains narrow, uniseriate, and elongate, terminating in a globular or ovoid cell with very dense contents. Repeated longitudinal divisions of the cells of the basal half result in an oval palea with an attenuated hair-like tip and bearing superficial and marginal club-shaped hairs (*Fig. 36*). The adult leaf bears uniseriate, multicellular, club-shaped hairs with a prominent globular terminal cell having dense dark contents (*Figs. 37-39*).

COMPARISON

Little is known regarding the gametophyte and much less about the young sporophyte of *Athyrium* and related genera, so much so that it is well nigh impossible to make many comparisons. Stokey (1951), Wagner (1952a) and others have shown the importance of characters of the gametophyte and the young sporophyte in assessing the phylogeny of the different genera of ferns. The author (Nayar, 1956) has shown the probable correlation between the gametophytic and sporophytic

hairs in some of the polypodiaceous ferns and has suggested that it may be true for other ferns also. The study of *A. esculentum* lends further support to this statement. The acicular hairs on the young sporophyte seem from present observations to be entirely new structures, the paleae and hairs being comparable to gametophytic trichomes in their ontogeny and fundamental morphology.

Thickening of the walls at the corners of the cells of the mature gametophytes was reported in *Athyrium filix-femina* (L.) Roth by Stokey (1951). *Athyrium esculentum* differs from this in having uniformly thickened walls. Unicellular, club-shaped hairs of the *A. esculentum* type occur in *A. angustifolium* (Michx.) Milde but are reported to be absent in *A. filix-femina* and *A. alpestre* Rylands (Stokey, 1951). Unicellular, club-shaped hairs with an apical cap, mixed with two- to three-celled, elongate, acicular hairs (comparable to the acicular hairs on young juvenile leaves of *A. esculentum*) occur profusely on the prothallus of *Tectaria* spp. (Kachroo, 1956) and *Cyclosorus* spp. (Kachroo, 1957). The development and morphology of the gametophytes in these genera also are comparable to those of *A. esculentum*.

The first juvenile leaves in the related genera of ferns (*Dryopteris*, *Polystichum*, etc.) are usually dichotomous in plan and four- to eight-lobed (Wagner, 1952a). The early juvenile leaves of *A. esculentum*, though dichotomous in plan, have a cuneate, almost entire lamina, resembling in some respects the first leaf of some species of *Asplenium* (Slosson, 1906). The early leaves of those Aspleniaceae that are known have a tendency toward a simple, single vein pattern in the simplest frond condition and generally a more or less obcordate shape and a dichotomously divided vein in the first several fronds (Wagner, 1952b). All of them possess the ability to produce dichotomous vein patterns up to at least 4 to 6 vein termini. Among the Aspidiaceae, *Tectaria* alone, as far as known, is comparable to *A. esculentum* in the form of the juvenile leaves.

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Recent Fern Literature

CENTRAL EUROPEAN FERNS.¹—In a collection of natural history books published by the Senckenberg Natural History Society, Georg Eberle has just written a book of high quality on the ferns of central Europe that is likely to attract new friends to these plants. It contains a summary on ferns in general—their organography and life cycle, a well-documented exposition on hybridity in central European ferns, taking account of the work of Manton and D. E. Meyer, an account of apogamy and apospory, and finally a treatment of the various species and their hybrids in the territory studied. The illustrations consist of two drawings and 92 magnificent photographs taken by the author between 1926 and 1958, showing the plants in their natural habitats.—A. LAWALRÉE.

¹ Eberle, Georg. *Farne im Herzen Europas*. pp. I-VIII, 1-116, ill. Obtainable from Verlag Dr. Waldemar Kramer, Frankfurt am Main, Germany, 1959. Price DM 8.50.